

# Influence Factors on the Formation of Acrylamide in the Amino Acid/Sugar Chemical Model System

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**Abstract** Acrylamide, a potential carcinogen and neurotoxin, could be formed during the heat processing of carbohydrate-rich foods under high temperatures. Maillard reaction between reducing sugars and amino acids is the main pathway to form acrylamide. All of the types of reducing sugars and amino acids as well as the addition of vitamins may influence the formation of acrylamide. To explore the influence of reducing sugars, amino acids and the addition of vitamins on acrylamide formation, different chemical model systems were designed and studied in this work. The results showed that the largest amount of acrylamide was produced in the asparagine/glucose model system. L-glycine, L-glutamic acid and L-cysteine caused different levels of reduction in the amounts of acrylamide produced in the asparagine/glucose model systems. Vitamin C and Vitamin B<sub>1</sub> strongly reduced the formation of acrylamide by more than 60% at the appropriate adding dosage of approximately 1%, whereas Vitamin B<sub>2</sub> and Vitamin B<sub>5</sub> only reduced the formation of acrylamide by 20-30% at the adding dosage of approximately 1%. However, Vitamin B<sub>2</sub> promoted the formation of acrylamide at adding dosages greater than 1%.

**Keywords:** *acrylamide, amino acids, reducing sugars, vitamins, food safety, chemical model system*

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## 1. Introduction

Food safety is a stake major issue, related to people's health and safety of life as well as economic development and social harmony. In recent years, the poisonous and harmful residues and contaminants in food, especially the hazardous substance produced in the food processing have been in hot research focus all over the world. In April 2002, researchers at the Swedish National Food Administration and Stockholm University for the first time found relatively high levels of acrylamide (AA) in the fried or baked potatoes and plant-based foods [1]. After the report was published, it immediately caused widespread concern in the world food industry. Many confirmatory experiments [2,3,4] have been performed by other research groups from academic, industrial and official laboratories. These investigations caused considerable concern as AA has been regarded as a compound that has neurotoxicity, reproductive toxicity, genetic toxicity and carcinogenicity in animals [5,6].

It is now generally accepted that the Maillard reaction between reducing sugars and carbonyl compounds at high temperatures is the key pathway for the formation of AA in foods [7,8,9,10,11]. Reducing sugars and amino acids involved in the Maillard reaction provide the molecular skeleton of AA. A large number of reports [12,13,14,15] have evaluated the effect of amino acids on AA, but few reports can be found on the effect of amino acid mixtures

on AA formation. Meanwhile, the type of reducing sugar is also an important factor that influences the formation of AA.

Many additives have been reported to influence AA formation. Several of these additives have inhibition effects, such as organic acids [16,17], chlorates [17,18,19], phenolic compounds [20,21], and plant extract [22,23]. In contrast, several of these additives have promoting effects, such as sucrose [24] and butylated hydroxytoluene [25]. Vitamins have traditionally been among the most widely applied chemical agents to enhance the nutritional values of food products. Moreover, flour itself contains many vitamins, including Vitamin B<sub>1</sub>, Vitamin B<sub>2</sub> and Vitamin B<sub>5</sub>, in addition to trace amounts of vitamin C. However, there is no scientific consensus about the influence of vitamins on AA formation in the amino acid/sugar reaction system.

Taking into consideration all described above, the present study aimed to investigate the influence factors on AA formation in the amino acid/sugar model system, especially the mixing of amide acids, the types of reducing sugars and the addition of vitamins. The tested reducing sugars included L-glucose (GLU), fructose (Fru), D-xylose (Xyl), maltose (Mal), D-anhydrous lactose (Lac) and galactose (Gal). The tested amino acids included L-asparagine (Asn), L-glycine (Gly), L-glutamic acid (Glu) and L-cysteine (Cys). The tested vitamins included L-ascorbic acid (vitamin C; VC), thiamin (vitamin B<sub>1</sub>; VB<sub>1</sub>), riboflavin (vitamin B<sub>2</sub>; VB<sub>2</sub>) and calcium pantothenate (vitamin B<sub>5</sub>; VB<sub>5</sub>).

## 2. Material and Methods

### 2.1. Reagents and chemicals

All solvents used were of HPLC grade, and the other chemicals were of analytical grade. Acrylamide (purity > 99%) and HPLC-grade methanol were obtained from J&K

Technology Co., Ltd. (Beijing, China). L-asparagine, L-glycine, L-glutamic acid, L-cysteine, L-glucose, fructose, D-xylose, maltose, D-anhydrous lactose, galactose, L-ascorbic acid, thiamin, riboflavin and calcium pantothenate were obtained from Hangzhou Mike Chemical Instrument Co., Ltd. (Hangzhou, China). The chemical structure formulas of the testing reducing sugars, amino acids and vitamins are listed in Table 1.

Table 1. Chemical structure formulas of the tested reducing sugars and amino acids

Type	Chemical structure formula	Type	Chemical structure formula	Type	Chemical structure formula
L-glucose (GLU)		L-asparagine (Asn)		L-ascorbic acid (VC)	
fructose (Fru)		L-glycine (Gly)		thiamin (VB1)	
D-xylose (Xyl)		L-glutamic acid (Glu)		riboflavin (VB2)	
galactose (Gal)		L-cysteine (Cys)		calcium pantothenate (VB5)	
Maltose (Mal)					
D-anhydrous lactose (Lac)					

### 2.2. Apparatus

An oven (Midea, China) was used in this experiment. The extracts were prepared with a solid phase extraction (Visiprep TM DL, Supelco, United States) with a HC-C18 solid phase extraction small column (500mg/3mL; Shanghai spectrum). Chromatographic analysis was performed on a high performance liquid chromatograph (Agilent 1260).

### 2.3. Chemical Model Reactions of AA Formation

Chemical model reactions were performed according to the method of [26] with minor modifications described by [27], and all of the experiments were performed in triplicate.

The amino acid/reducing-sugar model systems were prepared as follows: 0.1 mmol reducing sugars (GLU, Fru,

Xyl, Mal, Lac or Gal) and 0.1 mmol amino acids (Asn, Gly, Glu or Cys) were mixed with 3 mL of purified water. The reaction vials were heated in the oven at 160°C for 30 min, and the reaction mixtures were then diluted with purified water to 5 mL after they were cooled. The C18 solid phase extraction small columns were activated by 2 mL of methanol and 2 mL of water. The combined solvent (1 mL) was injected into the small column, and the effluent was discarded. The remaining portion of the combined solvent was also injected into the small column, and the effluent was collected for HPLC.

The dynamic Asn/reducing sugar model systems were prepared as follows: 0.1 mmol Asn and 0.1 mmol reducing sugars (GLU, Fru or Xyl) were mixed with 3 mL of purified water, and the reaction vials were heated in the oven at 160 °C for a certain gradient of time (5-50 min). Then, the reaction mixtures were treated as described above.

The mixed amino acid/GLU model systems were prepared as follows: 0.1 mmol amino acids were added to the Asn/GLU model system. The reaction vials were heated in the oven at 160°C for 30 min, and the reaction mixtures were then treated as described above.

The vitamin/Asn/GLU chemical model systems were prepared. (1) The four types of water-soluble vitamins (VC, VB<sub>1</sub>, VB<sub>2</sub> and VB<sub>5</sub>) were either added (1%) or not to the blank sample based on the Asn/GLU model system, and the reaction vials were heated in the oven at 160°C for 30 min. The reaction mixtures were then treated as described above. (2) The four types of water-soluble vitamins (VC, VB<sub>1</sub>, VB<sub>2</sub>, VB<sub>5</sub>) were either added (0.1-5%) or not to the blank sample based on the Asn/GLU model system, and the reaction vials were heated in the oven at 160°C for 30 min. The reaction mixtures were then treated as described above.

## 2.4. Analysis of AA by HPLC

The analysis method of AA was performed as described by [28] and [29]. This method was confirmed by HPLC in our study. The determination of AA formation was performed using a HPLC system (Agilent Technologies Co., USA) equipped with automatic sampler and diode array detector (DAD), and a reversed ZORBAX SB-C18ODSC18 (4.6×150 mm, 5 μm, Agilent Technologies Co., USA). Solution (15 μL) was injected into the column and eluted isocratically at 30°C with 10% methanol in water at a flow rate of 0.5 mL/min. The post running time was 15 min and the detection wavelength was 210 nm. AA was quantified by external calibration, in which the concentrations of AA standard solutions ranged from 0.01 to 1.0 μg/mL.

## 3. Results and Discussion

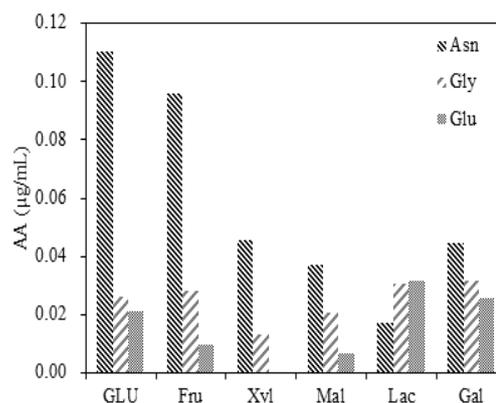
### 3.1. Effects of the Type of Reducing Sugars on AA Formation

Six types of reducing sugars, including four types of monosaccharide and two types of disaccharide, were examined for their effect on the formation of AA with three common amino acids in this experiment.

On one hand, different types of reducing sugars have different capacities to produce AA with amino acid. As indicated by [8], Asn is the main amino acid that reacts with reducing sugars to produce AA. As shown in Figure 1, different amounts of AA were produced in different Asn/reducing sugar model systems. The abilities of reducing sugars to produce AA with Asn were ranked as follows: GLU (0.1103 μg/mL) > Fru (0.0963 μg/mL) > Xyl (0.0459 μg/mL) > Gal (0.0449 μg/mL) > Mal (0.03751 μg/mL) > Lac (0.0175 μg/mL). This order indicates that monosaccharide may have a stronger capability to produce AA with Asn. In addition, different amounts of AA were produced in different Gly/reducing sugar model systems and Glu/reducing sugar model systems.

On the other hand, the same type of reducing sugars has different capacity to produce AA with different types of amino acids. In this experiment, GLU was selected and used, and three types of reducing sugar were examined for their effect on the formation of AA. As shown in Figure 1,

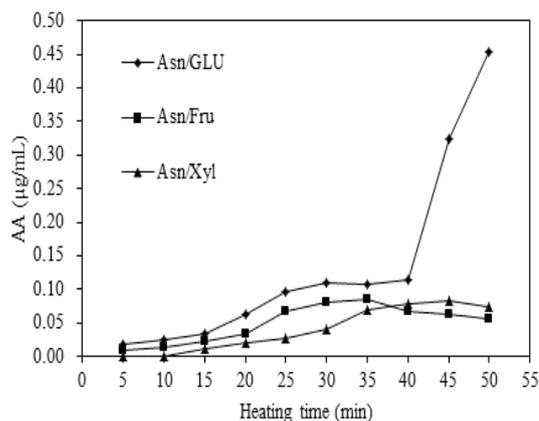
different amounts of AA were produced in different amino acid/GLU model systems. The abilities of GLU to produce AA with different types of amino acids were ranked as follows: Asn (0.1154 μg/mL) > Gly (0.0282 μg/mL) > Glu (0.0101 μg/mL).



**Figure 1.** Acrylamide formation in different amino acid/reducing sugar model systems

### 3.2. Dynamic of AA Formation in Asn/reducing Sugar Model Reaction Systems

To further investigate the effects of reducing sugar on acrylamide formation, the dynamics of three types of reducing sugar (GLU, Fru and Xyl) with asparagine in model reaction system were studied.

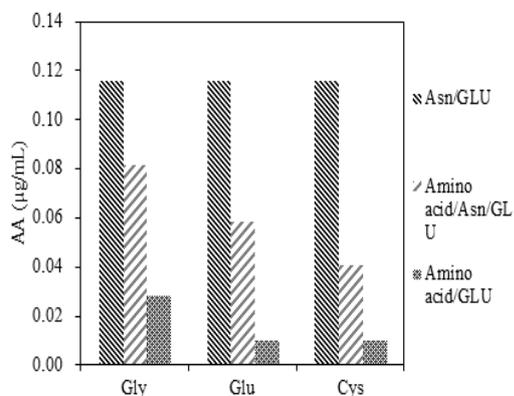


**Figure 2.** Acrylamide formation in three different dynamic asparagine/reducing sugar systems

As shown in Figure 2, the largest amount of AA among the three Asn/reducing sugar model systems was produced in the Asn/GLU dynamic system. In addition, the amount of AA increased with the extension of the heating time, and it reached a maximum at a certain heating time when the amount of AA decreased. For example, in the Asn/Fru system, the amount of AA increased with the extension of the heating time at 35 min from 0.0089 μg/mL to 0.0852 μg/mL, and then decreased to 0.0565 μg/mL in the next 15 min. Extraordinarily, there was a sudden increase of AA when the Asn/GLU model system was heated to an anhydrous state at 160°C for 40 min, which indicated that more AA is generated when the Maillard reaction is reacted in an anhydrous state.

### 3.3. Formation of AA in Mixed Amino Acid/GLU Model Systems

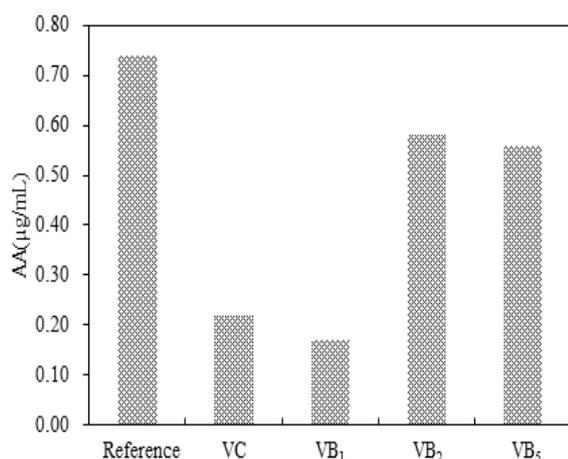
As shown in Figure 3, different amounts of AA were produced when three amino acids added to the Asn/GLU model system. The abilities of these three amino-acids to produce AA in mixed amino acid/GLU model systems were ranked as follows: Gly (0.0817  $\mu\text{g/mL}$ ) > Glu (0.0584  $\mu\text{g/mL}$ ) > Cys (0.0407  $\mu\text{g/mL}$ ).



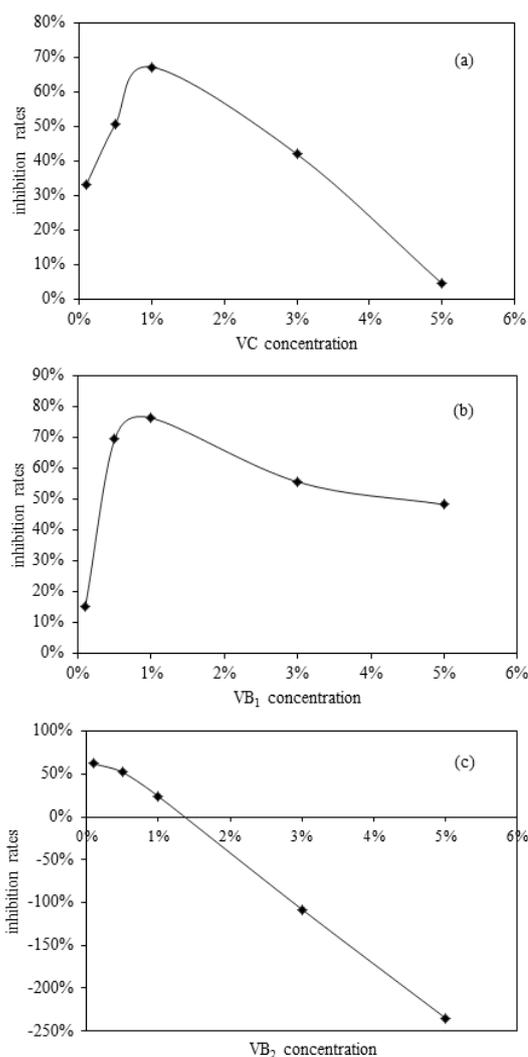
**Figure 3.** Formation of acrylamide in Asn/GLU model system, amino acid/GLU model systems and mixed amino acid/Asn/GLU model systems

Based on the amount of AA produced in the Asn/GLU model system, the addition of another type of amino acid reduced the formation of AA. The largest decrease in AA production (approximately 65%) occurred in the Cys/Asn/GLU model system, the second largest decrease in AA production (approximately 50%) occurred in the Glu/Asn/GLU model system. Moreover, the smallest decrease in AA production (approximately 30%) occurred in the Gly/Asn/GLU model system. The amount of AA produced in the amino acid/Asn/GLU model systems was less than that produced in the Asn/GLU model system. However, the amount of AA produced was higher than that produced in the amino acid/GLU model system. From a mechanistic point of view, it was proposed that the amount of AA produced in Gly/Asn/GLU model system might be the average of that produced in the Asn/GLU model system and the Gly/GLU model system, but the amount was greater. Therefore, a complex competition might occur between Asn and other amino acids.

### 3.4. Effects of Vitamin Additives on AA Formation



**Figure 4.** Effects of vitamin additives on the formation of acrylamide based on Asn/GLU model systems



**Figure 5.** Inhibition rates of (a) VC, (b) VB<sub>1</sub> and (c) VB<sub>2</sub> to acrylamide formation based on Asn/GLU model system

Based on the amount of AA produced in the Asn/GLU model system, the formation of AA was reduced by all of the tested water-soluble vitamins as shown in Figure 4. VC and VB<sub>1</sub> strongly reduced the formation of AA by more than 60%, whereas VB<sub>2</sub> and VB<sub>5</sub> only reduced the amount of AA formed by approximately 20-30%.

Different dosages of vitamins have different capacity to reduce the formation of AA as shown in Figure 5. Moreover, the inhibition rates of AA increased at the beginning followed by a reduction, and the rates peaked at approximately 1% under the adding concentration gradient of 0.1-5%, as shown in Figure 5 (a) and Figure 5 (b). Therefore, to maximize the AA formation inhibitory capacity of vitamins in the model system, the appropriate adding dosage of VC and VB<sub>1</sub> is approximately 1%. However, the inhibition rates of AA were in a downward trend, and they turned negative at approximately 1% under the added concentration gradient of 0.1-5%, as shown in Figure 5 (c). Consequently, the appropriate adding dosage of VB<sub>2</sub> is approximately within 1%, as VB<sub>2</sub> promoted the formation of AA at adding dosages greater than 1%.

## 4. Conclusions

In this study, the effect of amino acids, reducing sugars and vitamins on the formation of AA in the amino

acid/sugar chemical model system was systematically investigated. The results showed that different amino acid/sugar mixtures have different abilities to produce AA, and all of the tested vitamins were capable of inhibiting AA production. The largest amount of AA was produced in the Asn/GLU model system among the tested amino acid/reducing sugar model systems. Gly, Glu and Cys caused different levels of reduction in the amounts of AA, which may be due to the different degree of competition of Asn with other amino acids in the mixed amino acid/GLU model systems. VC and VB<sub>1</sub> strongly reduced the formation of AA by more than 60% at the appropriate adding dosage of approximately 1%, whereas VB<sub>2</sub> and VB<sub>5</sub> only reduced the formation of AA by 20-30% at the adding dosage of approximately 1%. In contrast, VB<sub>2</sub> promoted the formation of AA at adding dosages greater than 1%. Further studies are required to characterize the action mechanism of the vitamin additives that showed strong inhibitory effect against the formation of AA.

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## Statement of Competing Interests

The authors have no competing interests.

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